

Fig. 1

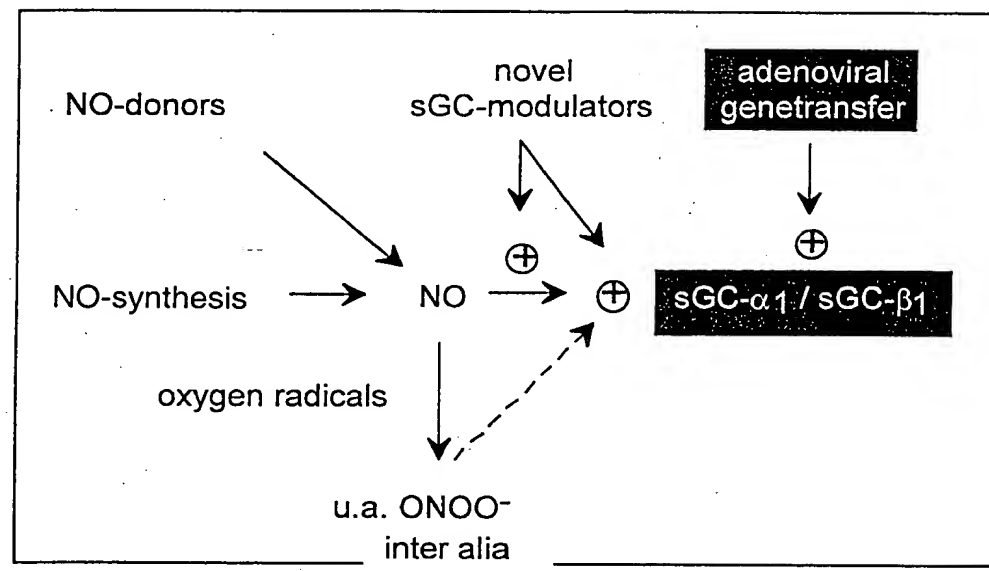


Figure 2

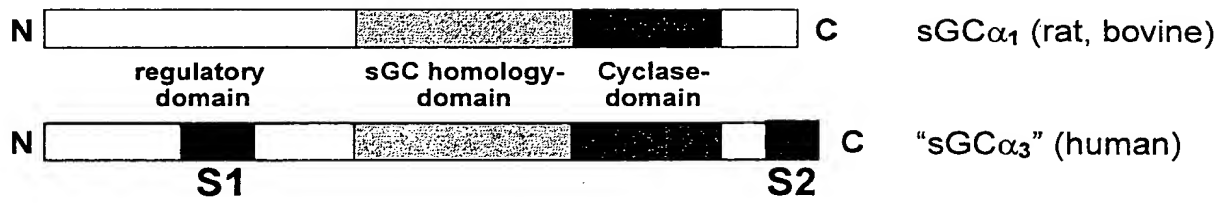
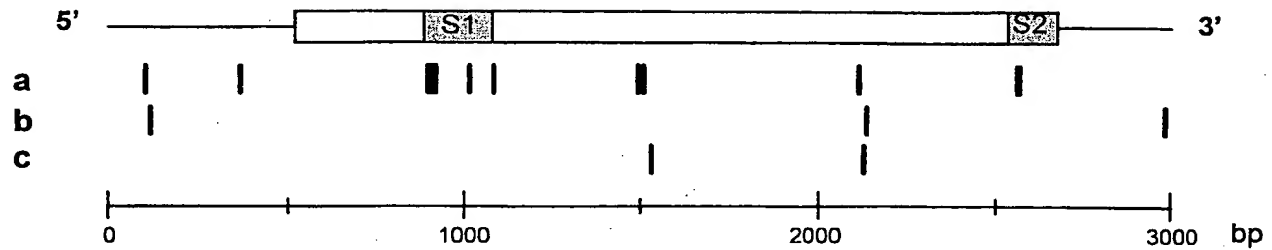


Figure 3

**a: nucleotide insertions**

C95, C367, T891, G900, T903, G913, T1006, G1074, G1487, A1488, A1489, G2108, G2555, T2560

b: nucleotide deletions

T between G111 and T112, T between T2128 and G2129, T between G2975 and T2976

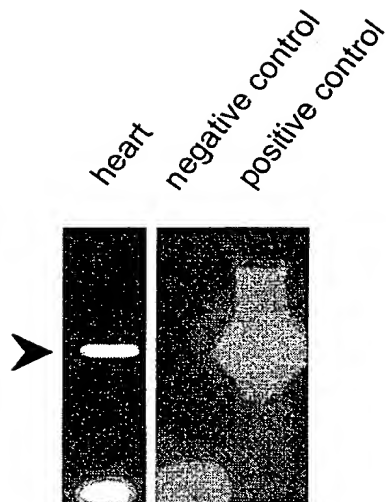
c: nucleotide exchanges

C1525>G, G2125>A

Figure 4

A

PCR
determination
of hsGC α 1



B

PCR
determination
of hsGC β 1

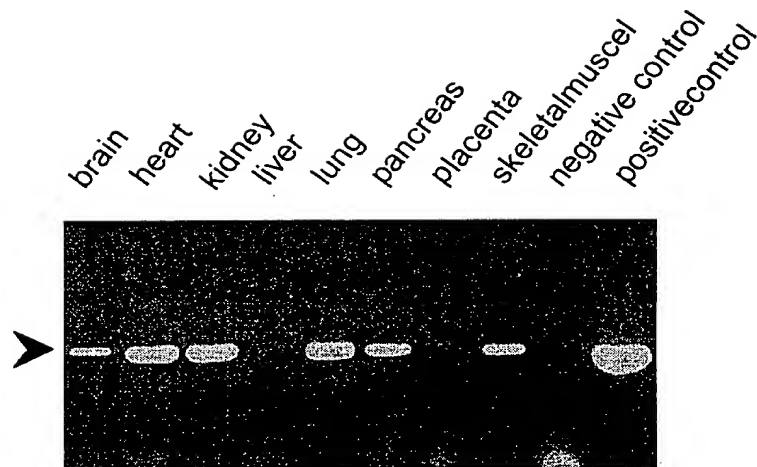
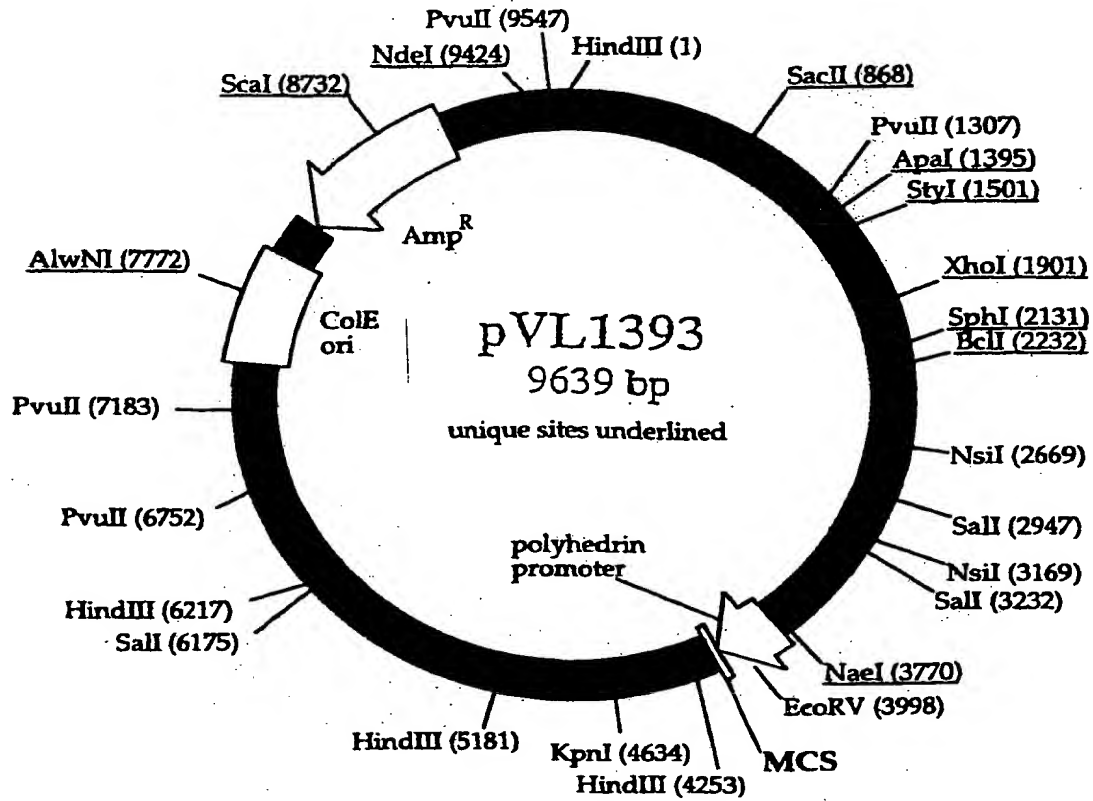


Figure 5

pVL1393 Baculovirus Transfer Vector



multiple cloning site (MCS) of pVL1393 with the unique restriction sites

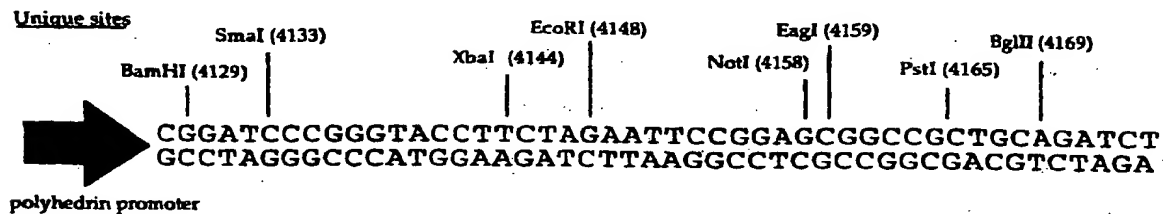
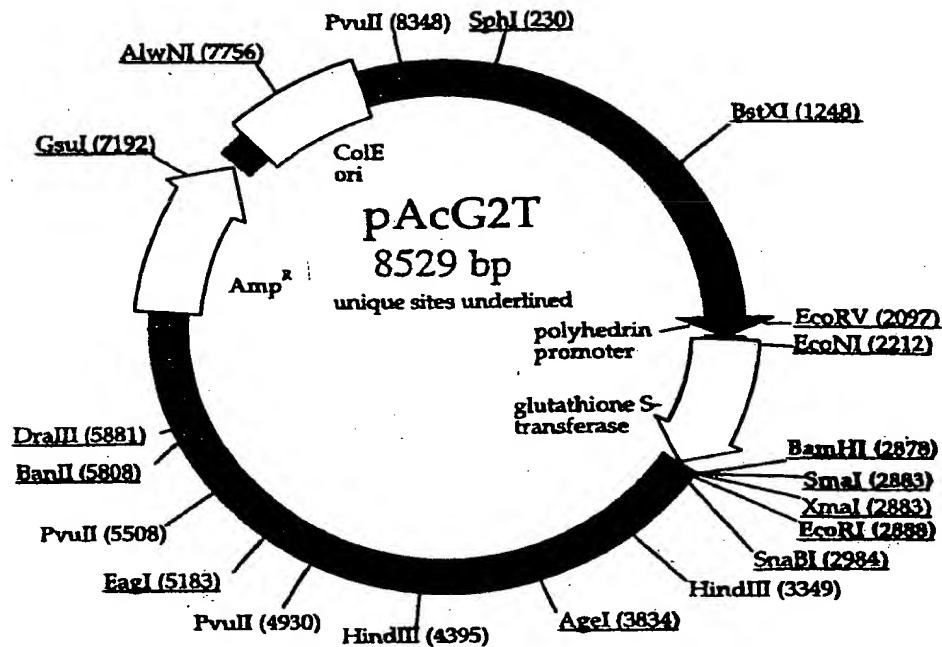


Figure 6

pAcG2T Baculovirus Transfer Vector



multiple cloning site (MCS) of pAcG2T downstream of glutathione-S-transferase sequence (GST) with the thrombin cleavage site and the unique restriction sites

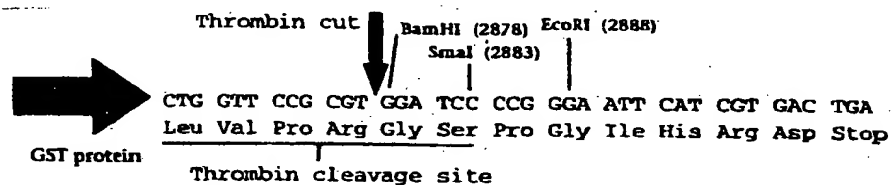
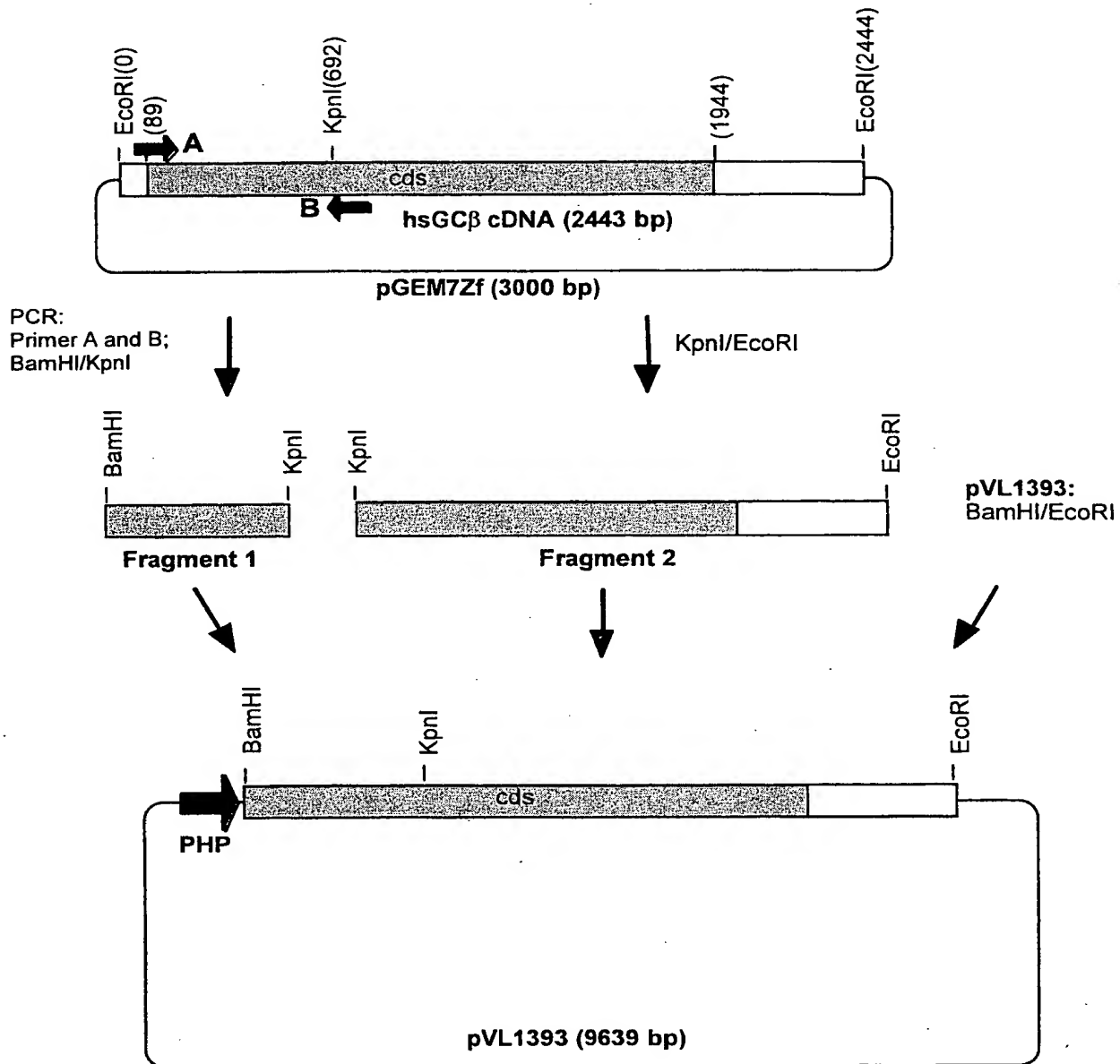
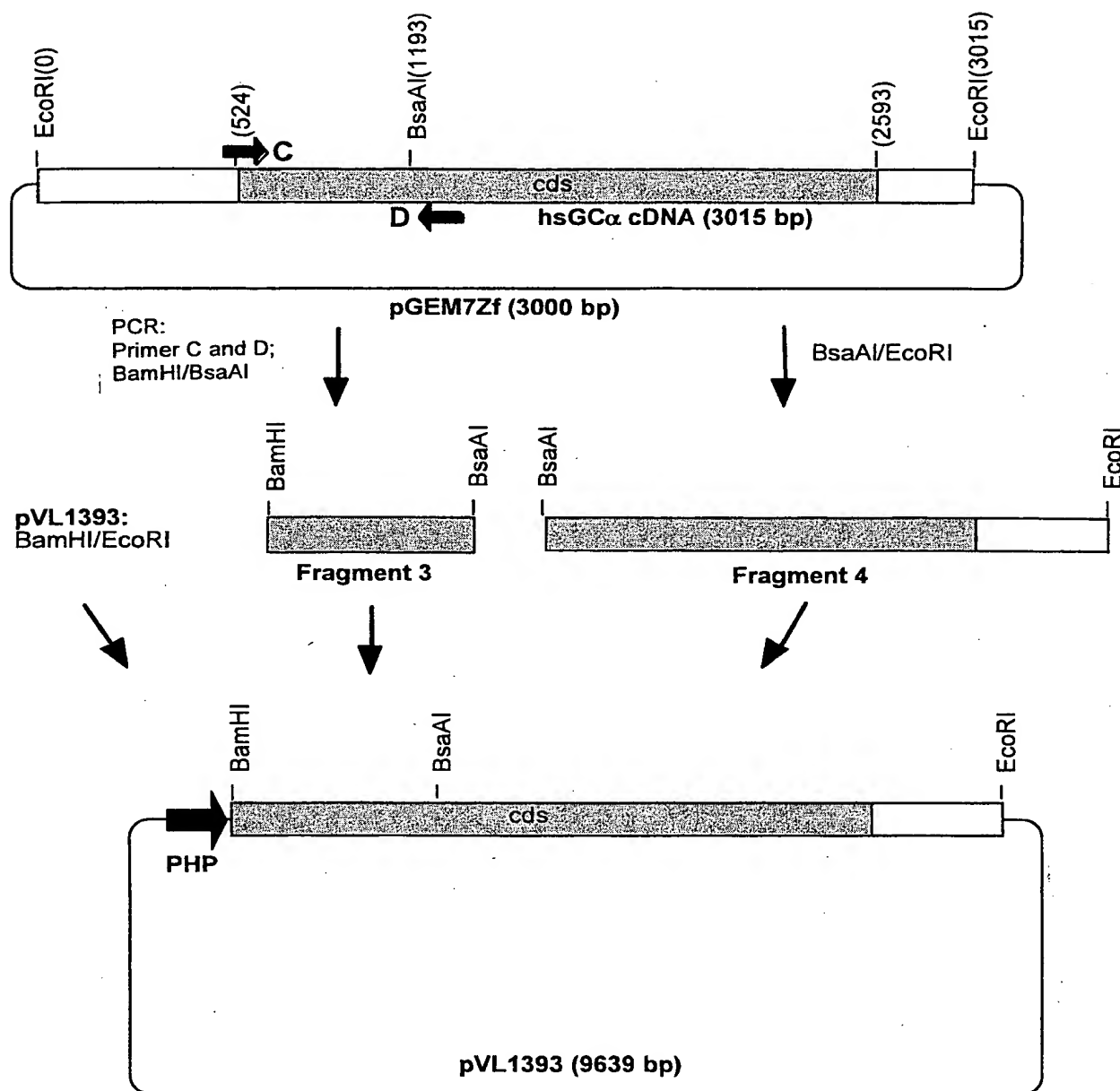


Figure 7: Cloning of hsGC β in pVL1393



Primer: A 5' AAAA **GGATCC** ATGTACGGATTTGTGAAT 3'
BamHI (89) (116)

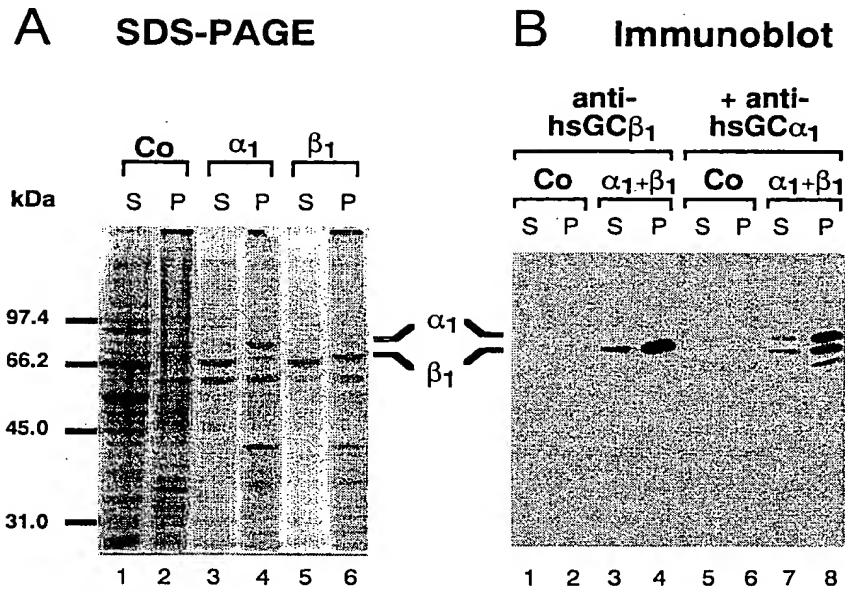
B 3' **CCATGG** GTCCTTAGTGCGTA 5'
(692) KpnI (711)

Figure 8: Cloning of hsGC α in pVL1393

Primer: C 5' AAAA GGATCC ATGTTCTGCACGAAGCTC 3'
BamHI (524) (541)

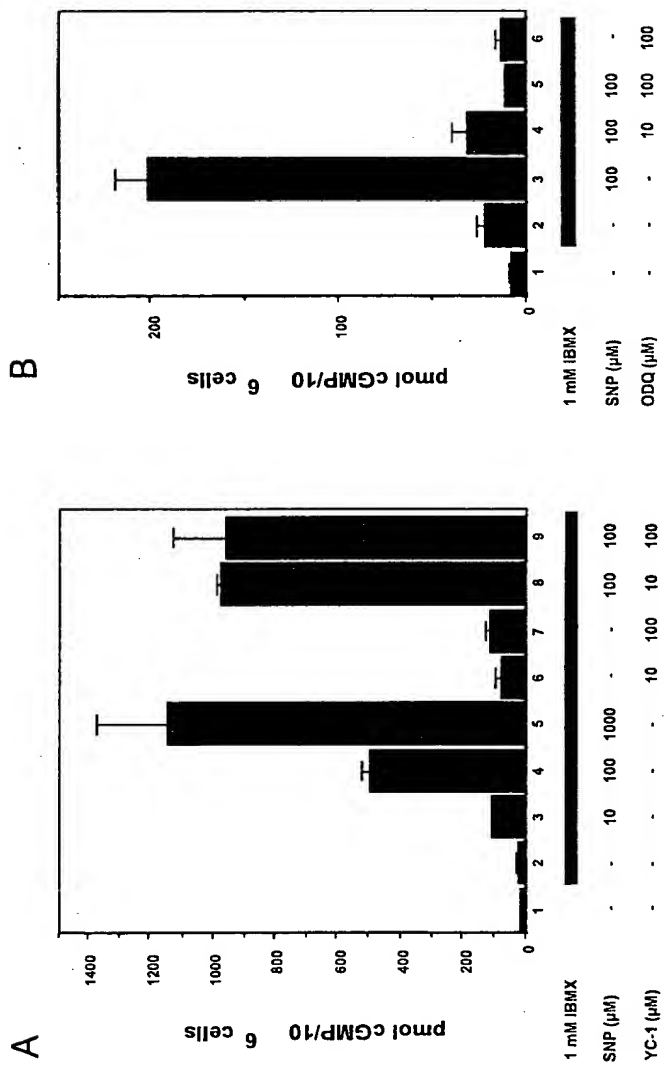
D 3' GGAGGGACGAAGGTATTA 5'
(1232) (1249)

Figure 9



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Fig. 10



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Fig. 11

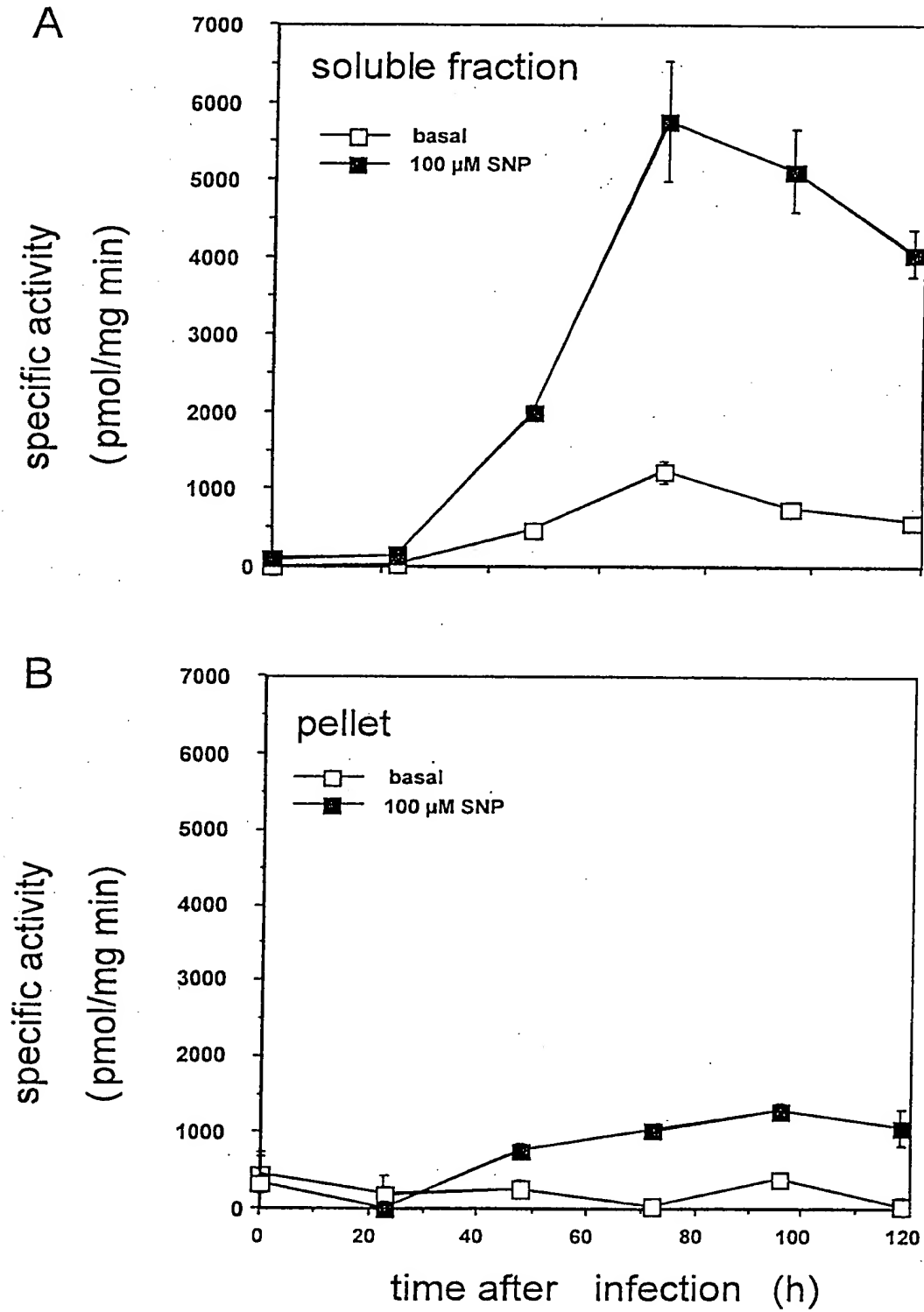


Figure 12: Purification of GST-hsGCalpha1/beta1 on GSH-Sepharose
4B

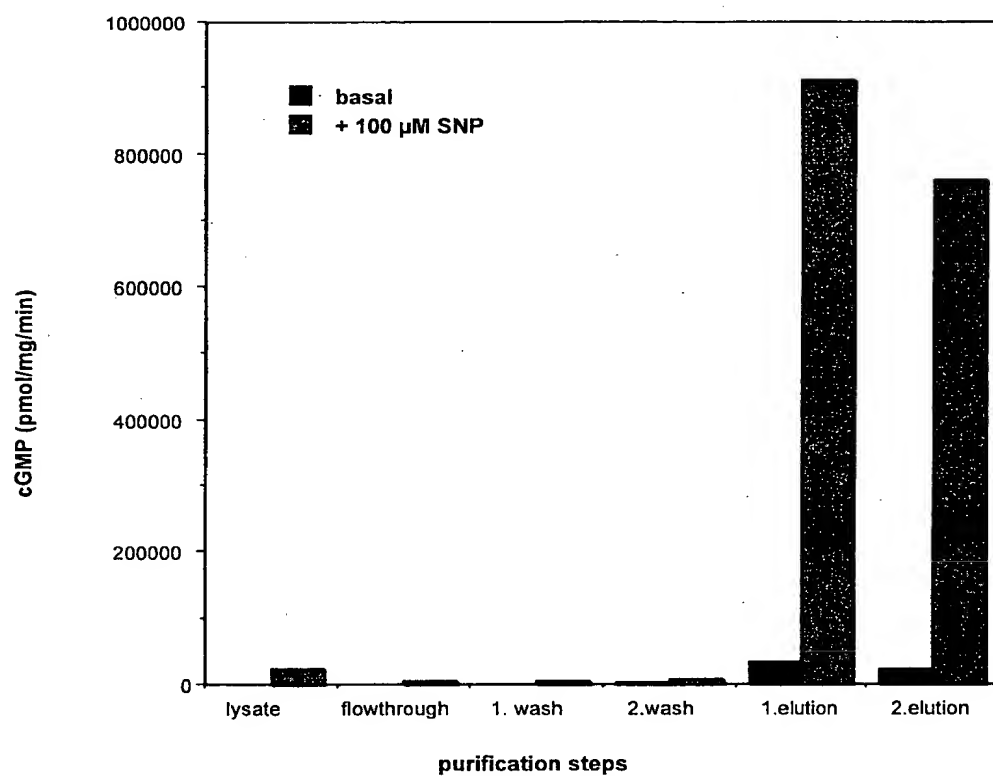


Figure 13

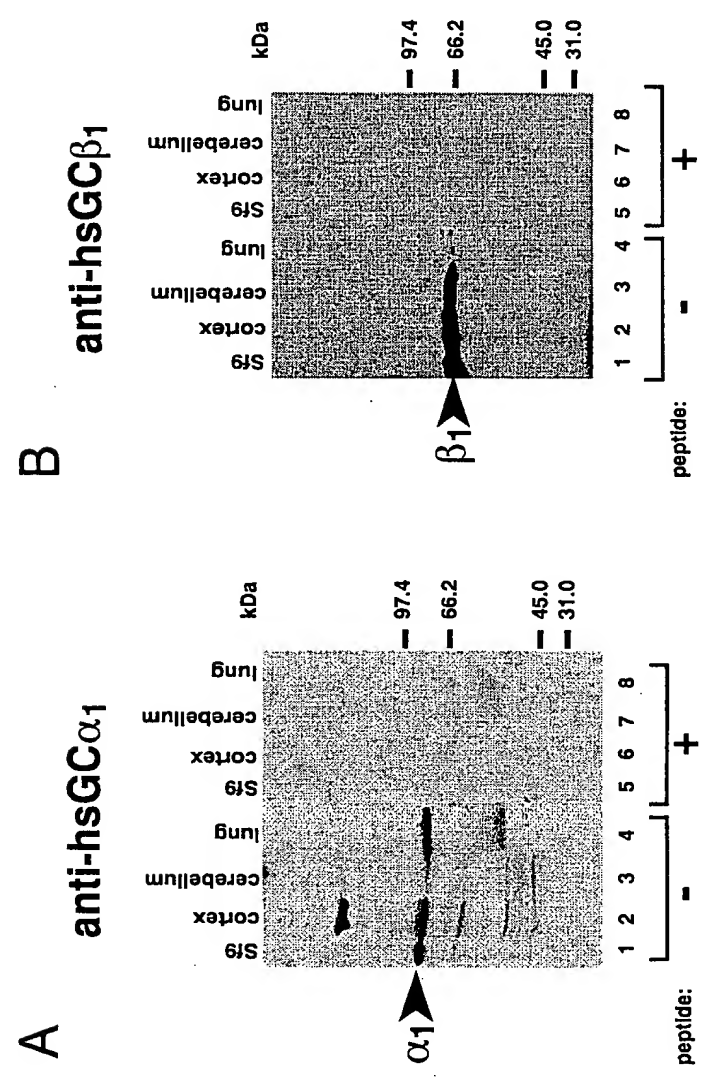


Figure 14

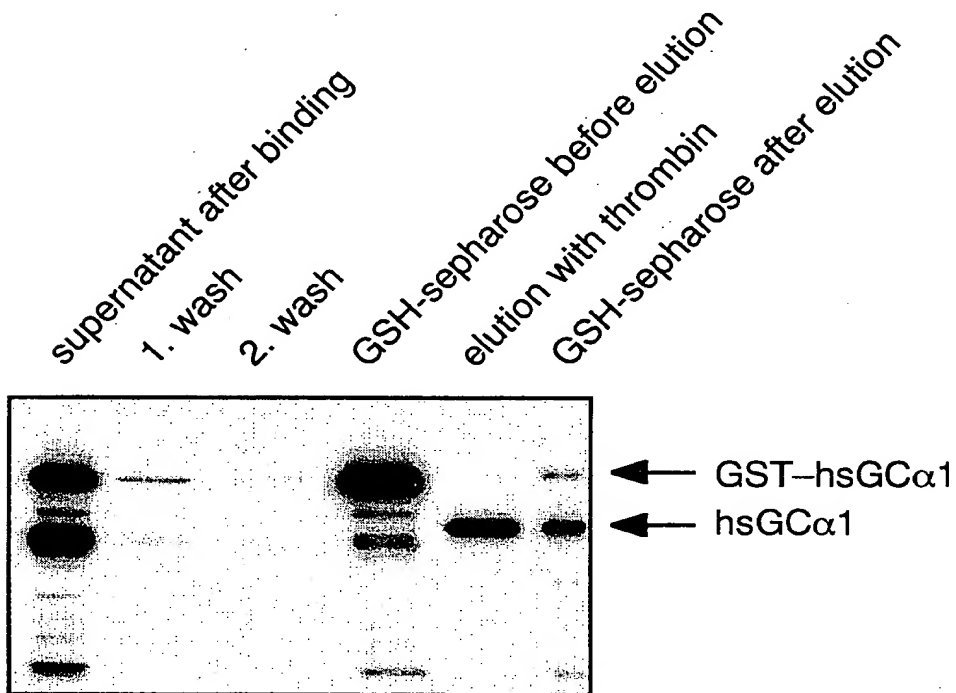


Figure 15: Purification of hsGC $\alpha 1/\beta 1$ in a Coomassie stained SDS polyacryamide gel

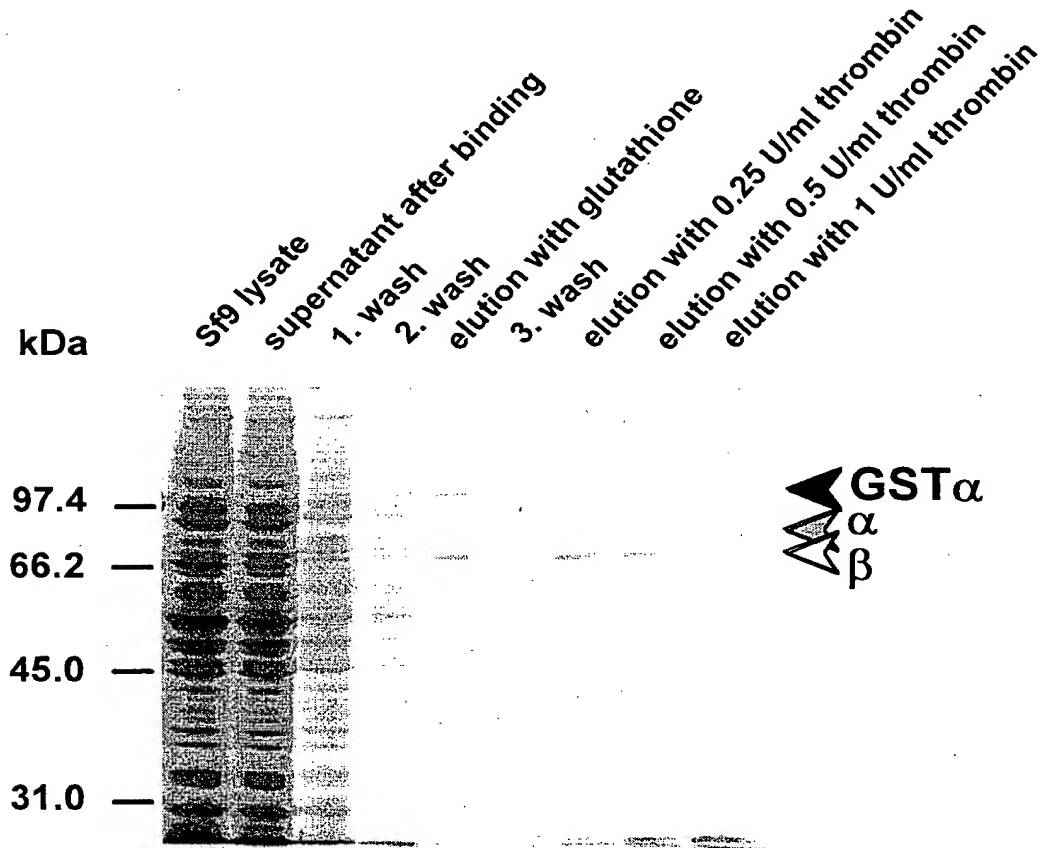


Figure 16: Construction of the hsGC-adenovectors

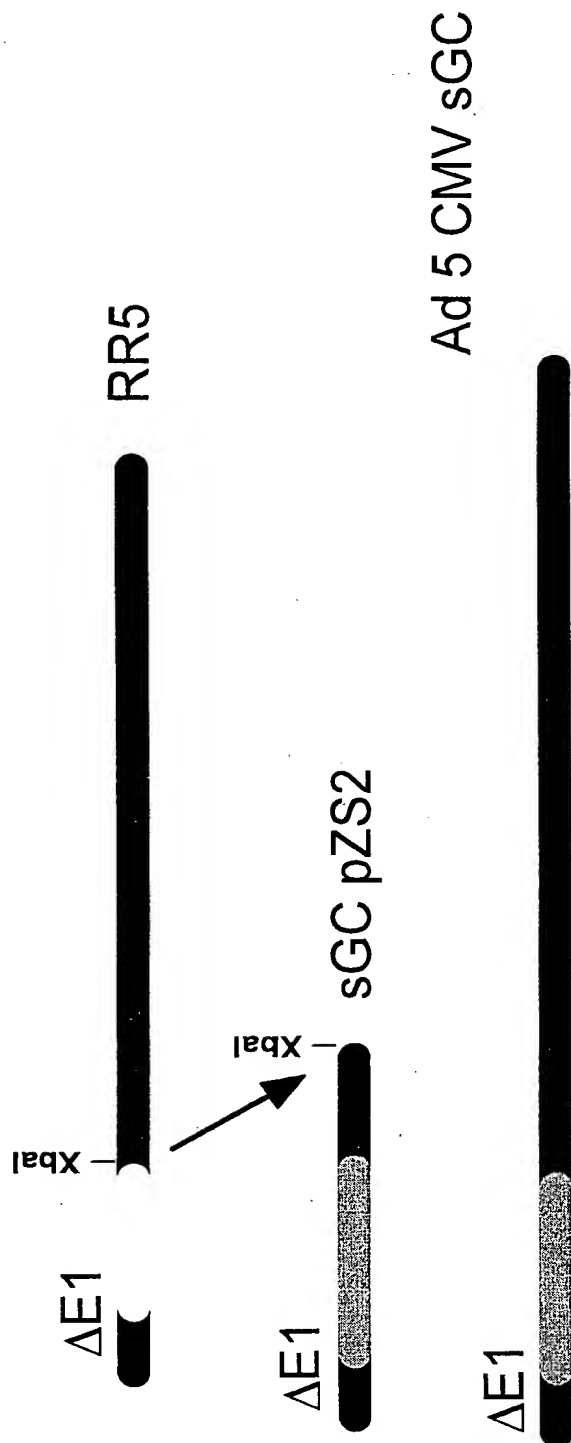


Figure 17:
Expression of human sGC in
adenovirus-infected EA.hy926
cells

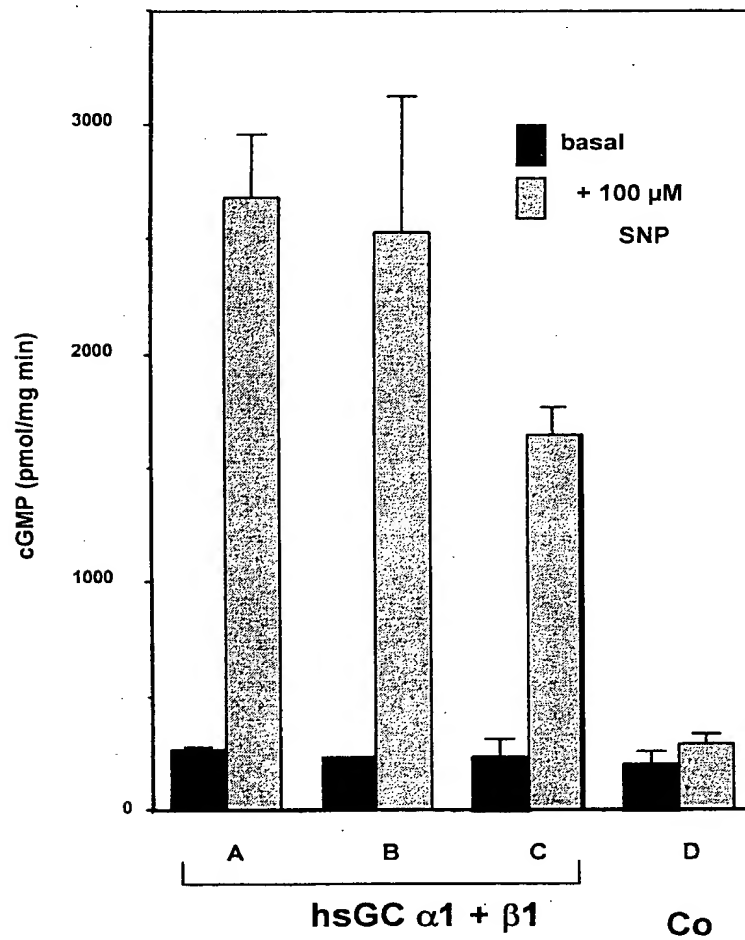


Figure 18

CCCTTATGGC	GATTGGGCGG	CTGCAGAGAC	CAGGACTCAG	TTCCCCTGCC	CTAGTCTGAG
CCTAGTGGGT	GGGACTCAGC	TCAGAGTCAG	TTTTCCAGAA	GCAGGTTTCA	GTGCAGAGTT
TTCCTACACT	TTTCCTGCGC	TAGAGCAGCG	AGCAGCCTGG	AACAGACCCA	GGCGGAGGAC
ACCTGTGGGG	GAGGGAGCGC	CTGGAGGAGC	TTAGAGACCC	CAGCCGGGCG	TGATCTCACC
ATGTGCGGAT	TTGCGAGGCG	CGCCCTGGAG	CTGCTAGAGA	TCCGGAAGCA	CAGCCCCGAG
GTGTGCGAAG	CCACCAAGAC	TGCGGCTCTT	GGAGAAAGCG	TGAGCAGGGG	GCCACCGCGG
TCTCCGCGCC	TGTCTGCACC	CTGTCGCCTG	AGCTGCCTGA	CAGTGACAAT	GACATCCCAG
TTACCAGTGT	CCTTGAATTG	ATAGTGGCTT	CTGTTTGTCA	GTCTCATATA	AGAACTACAG
CTCATCAGGA	GGAGATCGCA	GCAGGGTAAG	AGACACCAAC	ACCATGTTCT	GCACGAAGCT
CAAGGATCTC	AAGATCACAG	GAGAGTGTCC	TTTCTCCTTA	CTGGCACCAG	GTCAAGTTCC
TAACGAGTCT	TCAGAGGAGG	CAGCAGGAAG	CTCAGAGAGC	TGCAAAGCAA	CCGTGCCCCAT
CTGTCAAGAC	ATTCTTGAGA	AGAACATACA	AGAAAGTCTT	CCTCAAAGAA	AAACCAGTCG
GAGCCGAGTC	TATCTTCACA	CTTTGGCAGA	GAGTATTTGC	AAACTGATTT	TCCCAGAGTT
TGAACGGCTG	AATGTTGCAC	TTCAGAGAAC	ATTGGCAAAG	CACAAAATAA	AAGAAAGCAG
GAAATCTTTG	GAAAGAGAAG	ACTTTGAAAA	AACAATTGCA	GAGCAAGCAG	TTCAGCAGG
AGTTCAGTG	GAGGTTATCA	AAGAATCTCT	TGGTGAAGAG	GTTTTTAAAA	TATGTTACGA
GGAAGATGAA	AACATCCTTG	GGGTGGTTGG	AGGCACCCTT	AAAGATTTTT	TAAACAGCTT
CAGTACCCTT	CTGAAACAGA	GCAGCCATTG	CCAAGAAGCA	GGAAAAAGGG	GCAGGCTTGA
GGACGCCTCC	ATTCTATGCC	TGGATAAGGA	GGATGATTTT	CTACATGTTT	ACTACTTCTT
CCCTAAGAGA	ACCACCTCCC	TGATTCTTCC	CGGCATCATA	AAGGCAGCTG	CTCACGTATT
ATATGAAACG	GAAGTGGAAG	TGTCGTTAAT	GCCTCCCTGC	TTCCATAATG	ATTGCAGCGA
GTTTGTGAAT	CAGCCCTACT	TGTTGTACTC	CGTTCACATG	AAAAGCACCA	AGCCATCCCT
GTCCCCCAGC	AAACCCCAGT	CCTCGCTGGT	GATTCCCACA	TCGCTATTCT	GCAAGACATT
TCCATTCCAT	TTCATGTTTG	ACAAAGATAT	GACAATTCTG	CAATTTGGCA	ATGGCATCAG
AAGGCTGATG	AACAGGAGAG	ACTTTC AAGG	AAAGCCTAAT	TTTGAAGAAT	ACTTTGAAAT
TCTGACTCCA	AAAATCAACC	AGACGTTTAG	CGGGATCATG	ACTATGTTGA	ATATGCAGTT
TGTTGTACGA	GTGAGGAGAT	GGGACAACTC	TGTGAAGAAA	TCTTCAAGGG	TTATGGACCT
CAAAGGCCAA	ATGATCTACA	TTGTTGAATC	CAGTGCAATC	TTGTTTTTGG	GGTCACCCCTG
TGTGGACAGA	TTAGAAGATT	TTACAGGACG	AGGGCTCTAC	CTCTCAGACA	TCCCAATTCA
CAATGCACTG	AGGGATGTGG	TCTTAATAGG	GGAACAAGCC	CGAGCTCAAG	ATGGCCTGAA
GAAGAGGCTG	GGGAAGCTGA	AGGCTACCCT	TGAGCAAGCC	CACCAAGCCC	TGGAGGAGGA
GAAGAAAAAG	ACAGTAGACC	TTCTGTGCTC	CATATTTCCC	TGTGAGGTTG	CTCAGCAGCT
GTGGCAAGGG	CAAGTTGTGC	AAGCCAAGAA	GTTTCAGTAAT	GTCACCATGC	TCTTCTCAGA
CATCGTTGGG	TTCACTGCCA	TCTGCTCCCA	GTGCTCACC	CTGCAGGTCA	TCACCATTGCT
CAATGCACCTG	TACACTCGCT	TCGACCAGCA	GTGTGGAGAG	CTGGATGTCT	ACAAGGTGGA
GACCATTGGC	GATGCCTATT	GTGTAGCTGG	GGGATTACAC	AAAGAGAGTG	ATACTCATGC
TGTTTCAGATA	GCGCTGATGG	CCCTGAAGAT	GATGGAGCTC	TCTGATGAAG	TTATGTCTCC
CCATGGAGAA	CCTATCAAGA	TGCGAATTGG	ACTGCACTCT	GGATCAGTTT	TTGCTGGCGT
CGTTGGAGTT	AAAATGCCCC	GTTACTGTCT	TTTTTGAAAC	AATGTCACTC	TGGCTAACAA
ATTTGAGTCC	TGCAGTGTAC	CACGAAAAAT	CAATGTCAGC	CCAACAACCT	ACAGATTACT
CAAAGACTGT	CCTGGTTTCG	TGTTTACCCC	TCGATCAAGG	GAGGAACTTC	CACCAAACCT
CCCTAGTGAA	ATCCCCGGAA	TCTGCCATTT	TCTGGATGCT	TACCAACAAG	GAACAAACTC
AAAACCATGC	TTCCAAAAGA	AAGATGTGGA	AGATGGCAAT	GCCAATTTTT	TAGGCAAAGC
ATCAGGAATA	GATTAGCAAC	CTATATACCT	ATTTATAAGT	CTTTGGGGTT	TGACTCATTG
AAGATGTGTA	GAGCCTCTGA	AAGCACTTTA	GGGATTGTAG	ATGGCTAACA	AGCAGTATTA
AAATTTTCAGG	AGCCAAGTCA	CAATCTTTCT	CCTGTTTAAAC	ATGACAAAAAT	GTACTCACTT
CAGTACTTCA	GCTCTTCAAG	AAAAAAAAAA	AAACCTTAAA	AAGCTACTTT	TGTGGGAGTA
TTTCTATTAT	ATAACCAGCA	CTTACTACCT	GTA CTCAAAA	TTCAGCACCT	TGTACATATA
TCAGATAATT	GTAGTCAATT	GTACAAACTG	ATGGAGTCAC	CTGCAATCTC	ATATCCTGGT
GGAATGCCAT	GGTTATTAAA	GTGTGTTTGT	GATAGTGTCTG	TCAAAAAAAAA	AAAAAAAAAA
AAAAAAAAAA AAAAA					

Figure 19

Met Phe Cys Thr Lys Leu Lys Asp Leu Lys Ile Thr Gly Glu Cys Pro
 Phe Ser Leu Leu Ala Pro Gly Gln Val Pro Asn Glu Ser Ser Glu Glu
 Ala Ala Gly Ser Ser Glu Ser Cys Lys Ala Thr Val Pro Ile Cys Gln
 Asp Ile Pro Glu Lys Asn Ile Gln Glu Ser Leu Pro Gln Arg Lys Thr
 Ser Arg Ser Arg Val Tyr Leu His Thr Leu Ala Glu Ser Ile Cys Lys
 Leu Ile Phe Pro Glu Phe Glu Arg Leu Asn Val Ala Leu Gln Arg Thr
 Leu Ala Lys His Lys Ile Lys Glu Ser Arg Lys Ser Leu Glu Arg Glu
 Asp Phe Glu Lys Thr Ile Ala Glu Gln Ala Val Ala Ala Gly Val Pro
 Val Glu Val Ile Lys Glu Ser Leu Gly Glu Glu Val Phe Lys Ile Cys
 Tyr Glu Glu Asp Glu Asn Ile Leu Gly Val Val Gly Gly Thr Leu Lys
 Asp Phe Leu Asn Ser Phe Ser Thr Leu Leu Lys Gln Ser Ser His Cys
 Gln Glu Ala Gly Lys Arg Gly Arg Leu Glu Asp Ala Ser Ile Leu Cys
 Leu Asp Lys Glu Asp Asp Phe Leu His Val Tyr Tyr Phe Phe Pro Lys
 Arg Thr Thr Ser Leu Ile Leu Pro Gly Ile Ile Lys Ala Ala Ala His
 Val Leu Tyr Glu Thr Glu Val Glu Val Ser Leu Met Pro Pro Cys Phe
 His Asn Asp Cys Ser Glu Phe Val Asn Gln Pro Tyr Leu Leu Tyr Ser
 Val His Met Lys Ser Thr Lys Pro Ser Leu Ser Pro Ser Lys Pro Gln
 Ser Ser Leu Val Ile Pro Thr Ser Leu Phe Cys Lys Thr Phe Pro Phe
 His Phe Met Phe Asp Lys Asp Met Thr Ile Leu Gln Phe Gly Asn Gly
 Ile Arg Arg Leu Met Asn Arg Arg Asp Phe Gln Gly Lys Pro Asn Phe
 Glu Glu Tyr Phe Glu Ile Leu Thr Pro Lys Ile Asn Gln Thr Phe Ser
 Gly Ile Met Thr Met Leu Asn Met Gln Phe Val Val Arg Val Arg Arg
 Trp Asp Asn Ser Val Lys Lys Ser Ser Arg Val Met Asp Leu Lys Gly
 Gln Met Ile Tyr Ile Val Glu Ser Ser Ala Ile Leu Phe Leu Gly Ser
 Pro Cys Val Asp Arg Leu Glu Asp Phe Thr Gly Arg Gly Leu Tyr Leu
 Ser Asp Ile Pro Ile His Asn Ala Leu Arg Asp Val Val Leu Ile Gly
 Glu Gln Ala Arg Ala Gln Asp Gly Leu Lys Lys Arg Leu Gly Lys Leu
 Lys Ala Thr Leu Glu Gln Ala His Gln Ala Leu Glu Glu Lys Lys
 Lys Thr Val Asp Leu Leu Cys Ser Ile Phe Pro Cys Glu Val Ala Gln
 Gln Leu Trp Gln Gly Gln Val Val Gln Ala Lys Lys Phe Ser Asn Val
 Thr Met Leu Phe Ser Asp Ile Val Gly Phe Thr Ala Ile Cys Ser Gln
 Cys Ser Pro Leu Gln Val Ile Thr Met Leu Asn Ala Leu Tyr Thr Arg
 Phe Asp Gln Gln Cys Gly Glu Leu Asp Val Tyr Lys Val Glu Thr Ile
 Gly Asp Ala Tyr Cys Val Ala Gly Gly Leu His Lys Glu Ser Asp Thr
 His Ala Val Gln Ile Ala Leu Met Ala Leu Lys Met Met Glu Leu Ser
 Asp Glu Val Met Ser Pro His Gly Glu Pro Ile Lys Met Arg Ile Gly
 Leu His Ser Gly Ser Val Phe Ala Gly Val Val Gly Val Lys Met Pro
 Arg Tyr Cys Leu Phe Gly Asn Asn Val Thr Leu Ala Asn Lys Phe Glu
 Ser Cys Ser Val Pro Arg Lys Ile Asn Val Ser Pro Thr Thr Tyr Arg
 Leu Leu Lys Asp Cys Pro Gly Phe Val Phe Thr Pro Arg Ser Arg Glu
 Glu Leu Pro Pro Asn Phe Pro Ser Glu Ile Pro Gly Ile Cys His Phe
 Leu Asp Ala Tyr Gln Gln Gly Thr Asn Ser Lys Pro Cys Phe Gln Lys
 Lys Asp Val Glu Asp Gly Asn Ala Asn Phe Leu Gly Lys Ala Ser Gly
 Ile Asp End

Figure 20

CCCCCCCCCG CCGCTGCCGC CTCTGCCTGG GTCCCTTCGG CCGTACCTCT GCGTGGGGGC
TGCCTCCCCG GCTCCCGGTG CAGACACCAT GTACGGATTT GTGAATCACG CCCTGGAGTT
GCTGGTGATC CGCAATTACG GCCCCGAGGT GTGGGAAGAC ATCAAAAAAG AGGCACAGTT
AGATGAAGAA GGACAGTTTC TTGTCAGAAT AATATATGAT GACTCCAAAA CTTATGATTT
GGTTGCTGCT GCAAGCAAAG TCCTCAATCT CAATGCTGGA GAAATCCTCC AAATGTTTGG
GAAGATGTTT TTCGTCTTTT GCCAAGAATC TGGTTATGAT ACAATCTTGC GTGTCTGGG
CTCTAATGTC AGAGAATTTT TACAGAACCT TGATGCTCTG CACGACCACC TTGCTACCAT
CTACCCAGGA ATGCGTGAC CTTCTTTTAG GTGCACTGAT GCAGAAAAGG GCAAAGGACT
CATTTTGAC TACTACTCAG AGAGAGAAGG ACTTCAGGAT ATTGTCATTG GAATCATCAA
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TGAAGAATGT GATCATACTC AATTTTAAAT TGAAGAAAAA GAGTCAAAAAG AAGAGGATTT
TTATGAAGAT CTTGACAGAT TTGAAGAAAA TGGTACCCAG GAATCACGCA TCAGCCCATA
TACATTCTGC AAAGCTTTTC CTTTTCATAT AATATTTGAC CGGGACCTAG TGGTCACTCA
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CATCAATACT GTTTTTGTAT TGAGAAAGCAA GGAAGGATTG TTGGATGTGG AGAAATTAGA
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CTACTTACCT GAAGCAGATA GCATACTTTT TCTATGTTCA CCAAGTGTC TGAACCTGGA
CGATTTGACA AGGAGAGGGC TGTATCTAAG TGACATCCCT CTGCATGATG CCACGCGCGA
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CACATTGCTG TATTCTGTCC TTCCTCCGTC TGTGCCAAT GAGCTGCGGC ACAAGCGTCC
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TGCTTTCTGT AGCAAGCATG CATCTGGAGA AGGAGCCATG AAGATCGTCA ACCTCCTCAA
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CATGAAGGGC AAAAAAGAAC CAATGCAAGT TTGGTTTCTA TCCAGAAAAA ATACAGGAAC
AGAGGAAACA AAGCAGGATG ATGACTGAAT CTTGGATTAT GGGGTGAAGA GGAGTACAGA
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TAGTGTTCCA CATATATGTA TATGTATATT TTAATGACTA TAATGTAATA AAGTTTATAT
CATGTTGGTG TATATCATT TAGAAATCAT TTTCTAAAG AGT

Figure 21

Met Tyr Gly Phe Val Asn His Ala Leu Glu Leu Leu Val Ile Arg Asn
 Tyr Gly Pro Glu Val Trp Glu Asp Ile Lys Lys Glu Ala Gln Leu Asp
 Glu Glu Gly Gln Phe Leu Val Arg Ile Ile Tyr Asp Asp Ser Lys Thr
 Tyr Asp Leu Val Ala Ala Ala Ser Lys Val Leu Asn Leu Asn Ala Gly
 Glu Ile Leu Gln Met Phe Gly Lys Met Phe Phe Val Phe Cys Gln Glu
 Ser Gly Tyr Asp Thr Ile Leu Arg Val Leu Gly Ser Asn Val Arg Glu
 Phe Leu Gln Asn Leu Asp Ala Leu His Asp His Leu Ala Thr Ile Tyr
 Pro Gly Met Arg Ala Pro Ser Phe Arg Cys Thr Asp Ala Glu Lys Gly
 Lys Gly Leu Ile Leu His Tyr Tyr Ser Glu Arg Glu Gly Leu Gln Asp
 Ile Val Ile Gly Ile Ile Lys Thr Val Ala Gln Gln Ile His Gly Thr
 Glu Ile Asp Met Lys Val Ile Gln Gln Arg Asn Glu Glu Cys Asp His
 Thr Gln Phe Leu Ile Glu Glu Lys Glu Ser Lys Glu Glu Asp Phe Tyr
 Glu Asp Leu Asp Arg Phe Glu Glu Asn Gly Thr Gln Glu Ser Arg Ile
 Ser Pro Tyr Thr Phe Cys Lys Ala Phe Pro Phe His Ile Ile Phe Asp
 Arg Asp Leu Val Val Thr Gln Cys Gly Asn Ala Ile Tyr Arg Val Leu
 Pro Gln Leu Gln Pro Gly Asn Cys Ser Leu Leu Ser Val Phe Ser Leu
 Val Arg Pro His Ile Asp Ile Ser Phe His Gly Ile Leu Ser His Ile
 Asn Thr Val Phe Val Leu Arg Ser Lys Glu Gly Leu Leu Asp Val Glu
 Lys Leu Glu Cys Glu Asp Glu Leu Thr Gly Thr Glu Ile Ser Cys Leu
 Arg Leu Lys Gly Gln Met Ile Tyr Leu Pro Glu Ala Asp Ser Ile Leu
 Phe Leu Cys Ser Pro Ser Val Met Asn Leu Asp Asp Leu Thr Arg Arg
 Gly Leu Tyr Leu Ser Asp Ile Pro Leu His Asp Ala Thr Arg Asp Leu
 Val Leu Leu Gly Glu Gln Phe Arg Glu Glu Tyr Lys Leu Thr Gln Glu
 Leu Glu Ile Leu Thr Asp Arg Leu Gln Leu Thr Leu Arg Ala Leu Glu
 Asp Glu Lys Lys Lys Thr Asp Thr Leu Leu Tyr Ser Val Leu Pro Pro
 Ser Val Ala Asn Glu Leu Arg His Lys Arg Pro Val Pro Ala Lys Arg
 Tyr Asp Asn Val Thr Ile Leu Phe Ser Gly Ile Val Gly Phe Asn Ala
 Phe Cys Ser Lys His Ala Ser Gly Glu Gly Ala Met Lys Ile Val Asn
 Leu Leu Asn Asp Leu Tyr Thr Arg Phe Asp Thr Leu Thr Asp Ser Arg
 Lys Asn Pro Phe Val Tyr Lys Val Glu Thr Val Gly Asp Lys Tyr Met
 Thr Val Ser Gly Leu Pro Glu Pro Cys Ile His His Ala Arg Ser Ile
 Cys His Leu Ala Leu Asp Met Met Glu Ile Ala Gly Gln Val Gln Val
 Asp Gly Glu Ser Val Gln Ile Thr Ile Gly Ile His Thr Gly Glu Val
 Val Thr Gly Val Ile Gly Gln Arg Met Pro Arg Tyr Cys Leu Phe Gly
 Asn Thr Val Asn Leu Thr Ser Arg Thr Glu Thr Thr Gly Glu Lys Gly
 Lys Ile Asn Val Ser Glu Tyr Thr Tyr Arg Cys Leu Met Ser Pro Glu
 Asn Ser Asp Pro Gln Phe His Leu Glu His Arg Gly Pro Val Ser Met
 Lys Gly Lys Lys Glu Pro Met Gln Val Trp Phe Leu Ser Arg Lys Asn
 Thr Gly Thr Glu Glu Thr Lys Gln Asp Asp Asp end

Figure 22

Phe Thr Pro Arg Ser Arg Glu Glu Leu Pro Pro Asn Phe Pro

Figure 23

Lys Gly Lys Lys Glu Pro Met Gln Val Trp Phe Leu Ser Arg Lys Asn
Thr Gly Thr Glu Glu Thr

Figure 24

upper primer

AAAAGGATCC ATGTTCTGCA CGAAGCTC

lower primer

ATTATGGAAG CAGGGAGG

Figure 25

upper primer

AAAAGGATCC ATGTACGGAT TTGTGAAT

lower primer

ATGCGTGATT CCTGGGTACC

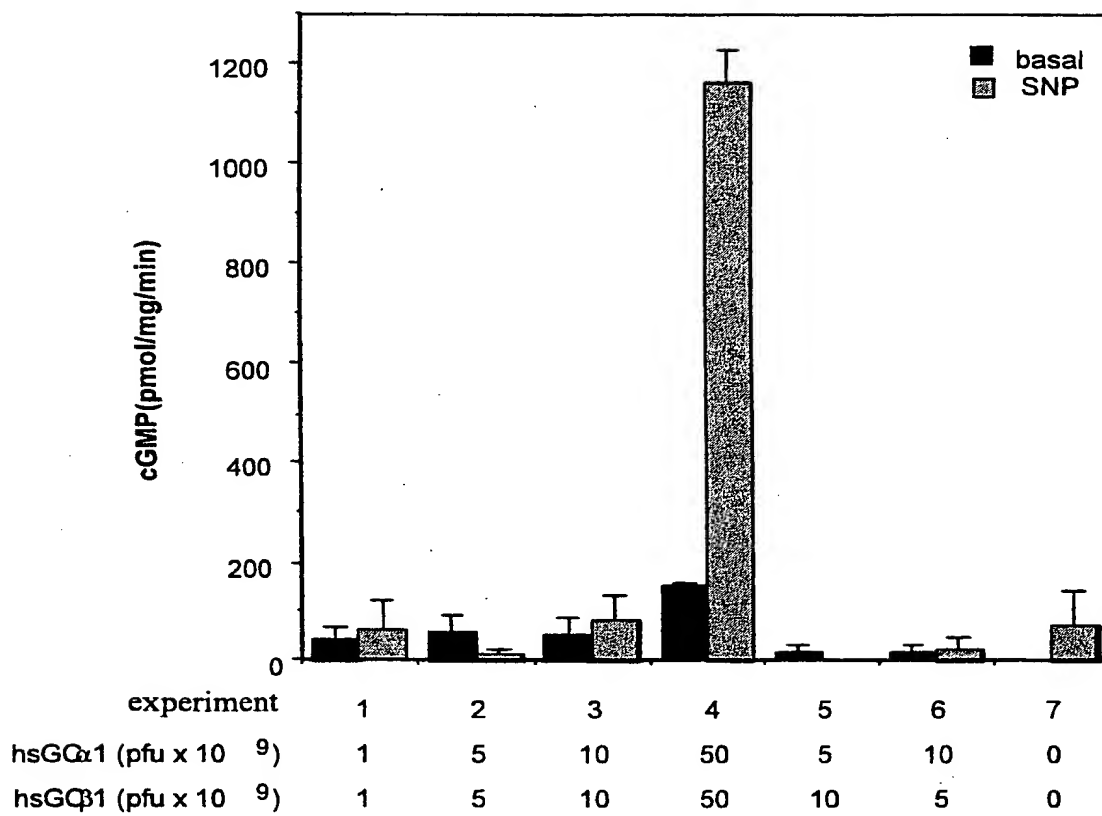
Figure 26

Figure 27

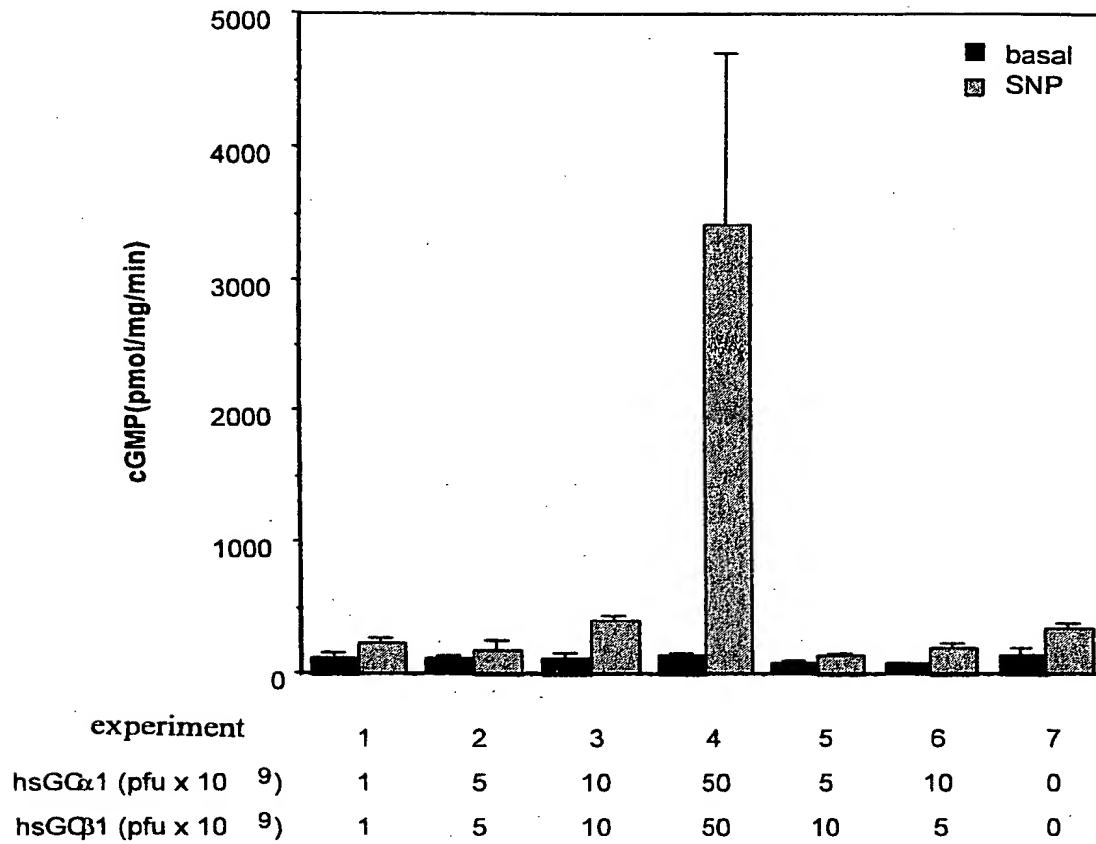


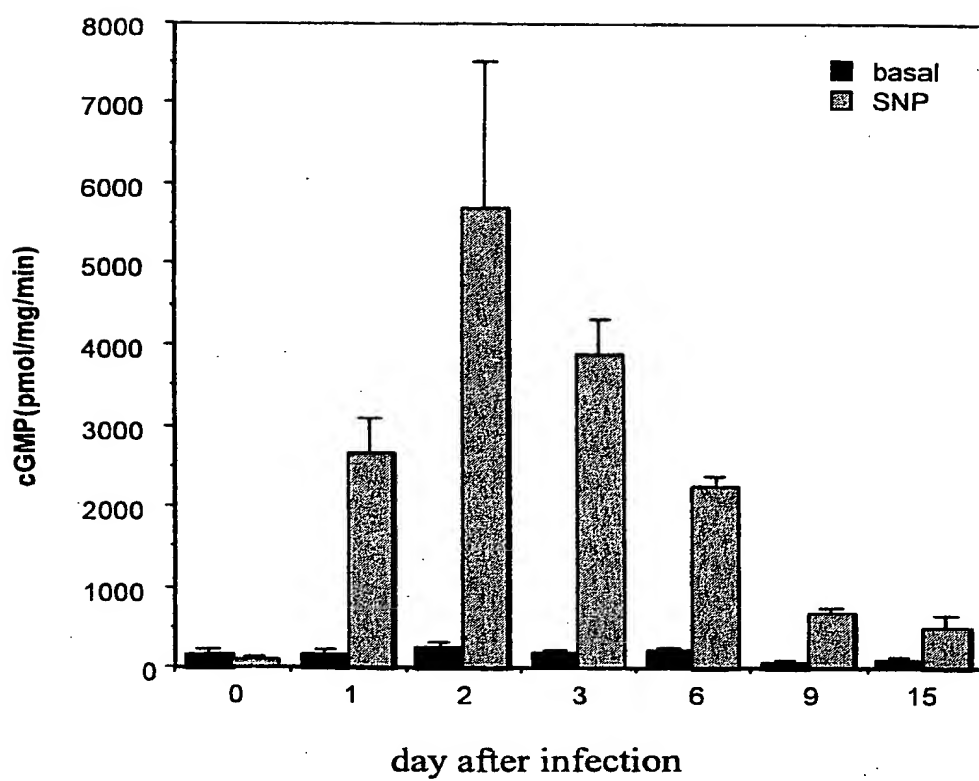
Figure 28

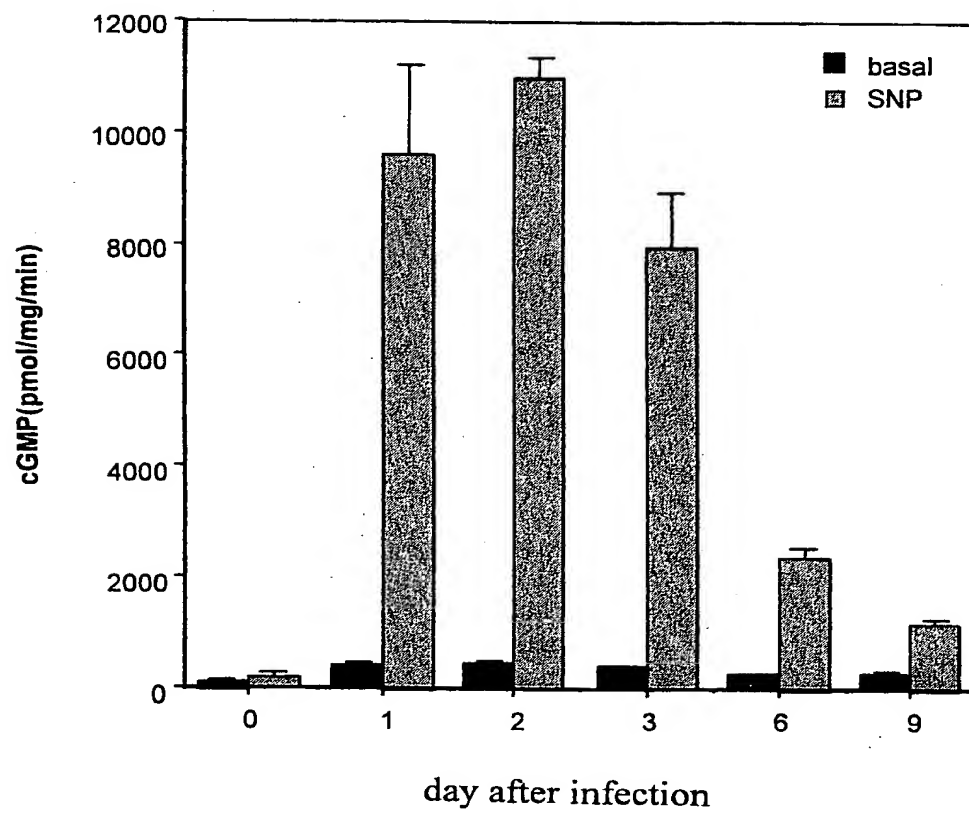
Figure 29

Figure 30

